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TITLE: UTILIZATION OF SIMIAN IMMUNODEFICIENCY VIRUS (SIV) INFECTION FOR SUBHUMAN PRIMATES TO EVALUATE EXPERIMENTAL CHEMOTHERAPY AND VACCINES

SUBTITLE: Titration of Two Strains of Simian Immunodeficiency Virus (SIV) in Macaca Mulatta and Tissue Culture

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1 July 1992 Final Report, Task I

Utilization of Simian Immunodeficiency Virus (SIV) Infection for Subhuman Primates to Evaluate Experimental Chemotherapy and Vaccines

Contract No. DAMD17-88-C-8160

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Subtitle: Titration of Two Strains of Simian Immunodeficiency Virus (SIV) in Macaca Mulatta and Tissue Culture

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The purpose of this research to to determine the infectivity of a biological clone (E11s) and a molecular clone (clone 8) of simian immunodeficiency virus (SIV) in Macaca Mulatta and tissue culture.

The study commenced on March 15, 1989 with the selection of the acceptable age and size of 35 candidate rhesus monkeys and housed in the study room. Daily health observations were recorded during the entire baseline period.

AIDS; BL3; Biotechnology; RA 1; Task I; Simian Immunodeficiency Virus; lab animals; monkey

Unclassified Unclassified Unclassified Unlimited

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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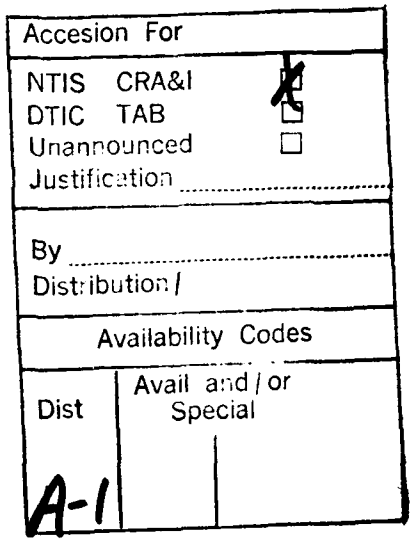

CONTRACT NO. DAMD 17-88-C-8160
FINAL REPORT
TASK ORDER I

Title:
Titration of Two Strains of Simian Immunodeficiency Virus (SIV) in Macaca mulatta and Tissue Culture.

Objective:
The purpose of this research is to determine the infectivity of a biological clone (E11s) and a molecular clone (clone 8) of simian immunodeficiency virus (SIV) in Macaca mulatta and tissue culture.

Study Design:
The study commenced on March 15, 1989 with the selection of the acceptable age and size of 35 candidate rhesus monkeys and housed in the study room. Daily health observations were recorded during the entire baseline period.

On September 27, 1989, all 35 monkeys were anesthetized, body weights and temperatures were recorded. Blood samples were taken from all animals for clinical evaluations (SMAC and CBC). Due to the aging of the 35 animals while waiting for the inoculum of SIV from the Department of the Army, the animals were returned to the colony on November 12, 1989. The next day (November 13, 1989) 21 rhesus monkeys meeting age and size requirements were selected and housed in the study room. These animals were observed daily throughout the entire baseline period. Health records including body weights, body temperature, heart beats, as well as clinical evaluations (SMAC & CBC) were recorded twice each month.

The E11s inoculum was received from the Department of the Army in May, 1990. Serum from each animal was collected and frozen for serologic evaluation (Western blots) as baseline control before the inoculation of E11s on May 15, 1990.

Experimental Design:

1. Animal Inoculations:

Animals were inoculated by Dr. Mikulas Popovic on May 15, 1990. Each animal received 1 ml of viral inoculum unless noted otherwise on the following table:

Log 10 Dilution of Inoculum	Number on Inoculum Tube	No. of Animals per Group	Animal Designations
10^{-4}	0503678	3	93D, 107D, 67D
10^{-5}	0503679	4	*111D, 35D, *9D, 1D
10^{-6}	0503680	4	*87D, 94D, 3D, 83D
10^{-7}	0503719	4	*49D, 20D, 99D, 101D
10^{-8}	0503720	3	108D, 26D, 38D
10^{-9}	0503721	3	39D, 30D, 103D
NO VIRUS	-	1	116D

***Between 0.9 ml and 1 ml per animal**

In addition to the cocultivation and immunoblots at PRI, a schedule for shipments of whole blood specimens from the monkeys to be sent to various collaborators via overnight delivery was provided by the Department of the Army as follows:

Specimen Day of Arrival	PRI Cocult	PRI Western Blot	NCI Specimen	Jackson Specimen
Day 0, 5/16/90			6-10 ml	
Week 4, 6/13/90		X	6-10 ml	from RB
Week 6, 6/27/90	X	X	none	none
Week 9, 7/18/90			4 ml	4 ml
Week 15, 8/28/90			4 ml	6 ml
Week 18, 9/18/90 (4th month)	X	X	none	none
Week 21, 10/21/90			none	6-10 ml
Week 26, 11/13/90		X	10 ml	10 ml

2. Virus Isolation I (under the direction of Dr. Suzanne Gartner)

Methods:

At week 7 post inoculation, virus isolation was performed. Peripheral blood mononuclear cells (PBMC) were separated from heparinized whole monkey blood using ficoll-hypaque gradients. PBMC were cultured in complete RPMI [1640 supplemented with 20% Fetal Bovine Serum, 20 μM L-glutamine, penicillin (100u/ml) - streptomycin (100 mcg/ml)], and 5μcg/ml PHA-P (Difco Co.). On day 3, the monkey cells were cocultivated with normal human lymphoblasts. (The human cells had been cryopreserved and were stimulated for three days with PHA-P prior to use). One x 10^7 monkey cells were cocultured with $5x10^6$ human cells in complete RPMI supplemented with 10% Interleukin 2 (Boeringer-Mannheim). Culture fluids were harvested at days 3, 6, 11, 14, 17, 21, 24, and 27 and assayed for reverse transcriptase (RT) activity. In addition, as a "last chance" attempt to recover virus, $5x10^6$ additional normal lymphoblasts (3 days post PHA-P stimulation) were added to each ongoing cocultivation on day 17, since our previous experience in the human system suggested that this is sometimes a way to "boost" low level virus expression into the range detectable by RT.

Results:

The results are summarized in the table below. At this time point, virus was unequivocally isolated from only one animal, #67D, which received the highest concentration of virus (10^{-4}). Cocultures from this animal were positive for RT activity at 5 different time points. In some instances, very borderline RT activity was detected at only one time point in some other cultures. These cultures were considered negative for virus detection since the addition of fresh target cells at a presumably optimal time did not result in detectable amplication of virus expression.

VIRUS ISOLATION AT WEEK 7 POST INOCULATION

Inoculum	Animal	Isolation
10^{-4}	93D	-
10^{-4}	107D	-
10^{-4}	67D	+
10^{-5}	111D	-
10^{-5}	35D	-
10^{-5}	9D	-
10^{-5}	1D	-
10^{-6}	87D	-
10^{-6}	94D	-
10^{-6}	3D	-
10^{-6}	83D	-
10^{-7}	49D	-
10^{-7}	20D	-
10^{-7}	99D	-
10^{-7}	101D	-
10^{-8}	108D	-
10^{-8}	26D	-
10^{-8}	38D	-
10^{-9}	39D	-
10^{-9}	30D	-
10^{-9}	103D	-
Uninfected Control	116D	-

Virus Isolation II (under the direction of Dr. Preston A. Marx):

Methods:

According to the results from the Western blots at week 18, monkeys 93D, 107D, and 67D (10^{-4} viral inoculum) had antibodies to p28, p32, p60, p66, and gp120. Similarly, animal 1D (10^{-5} inoculum) had antibodies to each of these proteins. These animals along with 3D (10^{-6} inoculum) and 116D (uninfected control) were chosen to use for the virus isolation for confirmation. Peripheral blood mononuclear cells (PBMC) were separated from heparinized whole blood using lymphocyte separation medium (LSM)

gradients. The monkey cells were cocultured with CEMx174 cells in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum, 100μM L-glutamine, penicillin (100u/ml) - streptomycin (100 mcg/ml). Supernatant (1 ml) from each coculture was taken weekly and frozen for RT assay. The cell cultures were maintained for eight weeks. Results showed no detectable RT activity in the cocultures. This may suggest that the CEMx174 cell line may not be an appropriate cell line for growth of the SIV E11s isolate, however further work is needed.

3. Serologic Evaluation Methods:

At 4, 7, 18, and 26 weeks post inoculation (June 13th, July 3rd, September 18th, and November 13th respectively) serum samples were obtained from the animals for serologic evaluation using Western blot analysis. A brief description of the method used is as follows:

The Protoblot Western Blot AP system (Promega) used for the serologic evaluation of monkey sera is a rapid, sensitive detection of proteins immobilized on nitrocellulose membrane (0.45μ) transferred from 7-20% gradient PAGE after electrophoresis. This method is based on the enzyme-linked immunodetection of antigen specific antibodies (monkey sera) using goat anti-human -IgG as second antibodies conjugated with alkaline phosphatase (AP). Following three hours of incubations with the monkey sera (primary antibody) and thirty minutes incubations with the anti-human IgG alkaline phosphatase conjugate, a solution containing the color development substrate is added (NBT: nitro blue tetrazolium, 50mg/ml in 70% dimethylformamide and BCIP:5-bromo-4-chloro-3-indolyl phosphate, 50 mg/ml in dimethyl formamide). Sites of antigen localization turn a dark purple color as the result of alkaline phosphatase.

Results:

The week 18 and 26 results are briefly summarized in the table below. The entire findings (weeks 4, 7, 18, and 26) are summarized in TABLE 1.

SEROCONVERSION DETERMINATION AT 18 AND 26 WEEKS POST INOCULATION

Inoculum	Animal	Seroconversion
10^{-4}	93D	+
10^{-4}	107D	+
10^{-4}	67D	+
10^{-5}	111D	-
10^{-5}	35D	-
10^{-5}	9D	-
10^{-5}	1D	+
10^{-6}	87D	-
10^{-6}	94D	-
10^{-6}	3D	-
10^{-6}	83D	-
10^{-7}	49D	-
10^{-7}	20D	-
10^{-7}	99D	-
10^{-7}	101D	-
10^{-8}	108D	-
10^{-8}	26D	-
10^{-8}	38D	-
10^{-9}	39D	-
10^{-9}	30D	-
10^{-9}	103D	-
Uninfected Control	116D	-

TABLE 1

Dil.	Animal #	4 Weeks after Inoculation					7 Weeks after Inoculation					18 Weeks after Inoculation					26 Weeks after Inoculation				
		gp120	p66	p60	p32	p28	gp120	p66	p60	p32	p28	gp120	p66	p60	p32	p28	gp120	p66	p60	p32	p28
10^{-4}	93D	-	+/-	-	-	-	+	-	-	+	-	+	+	+	+	+	+/-	-/-	-/-	+	+
10^{-4}	107D	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-4}	67D	+/-	+	+/-	-	-	+	+/-	-/-	+	+/-	+	+	+	+	+	+	+/-	+/-	+	+
10^{-5}	111D	-	-	-	-	-	-	+	-	-	-	+/-	+/-	-	-	-	-	-	-	-	-
10^{-5}	35D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-5}	9D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-5}	1D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10^{-6}	87D	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
10^{-6}	94D	+/-	-	+	-	-	+/-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-6}	3D	+/-	-	+	-	-	+/-	+/-	+/-	-	-	+/-	-	-	-	-	-	-	-	-	-
10^{-6}	83D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-7}	49D	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-7}	20D	-	+/-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-7}	99D	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-7}	101D	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+/-
10^{-8}	108D	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-8}	26D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-
10^{-8}	38D	-	-	+/-	-	-	-	+/-	+/-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-9}	39D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10^{-9}	30D	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10^{-9}	103D	-	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
-C	(116D)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+C		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

By week 18, animals 93D, 107D, and 67D (10^{-4} viral inoculum) had antibodies to p28, p32, p60, p66, and gp120. Similarly, animal 1D (10^{-5} viral inoculum) also had antibodies to each of these proteins. The week 26 Western blots also showed similar results from these four animals. Based on these data, only these four animals can be considered to have seroconverted.

At weeks 4 and 7, animals 107D (10^{-4} inoculum) and 1D (10^{-5} inoculum) had antibodies to each of the viral proteins. Whereas animals 93D and 67D (10^{-4} inoculum) lacked antibodies to some of the viral proteins at these earlier time points.

It should be noted that at week 26 animals 93D and 67D (10^{-4} inoculum) showed weakened antibody response to p60, p66, and gp120 compared to the Western blots at week 18. Also, animals 101D (10^{-7} inoculum) and 103D (10^{-9} inoculum) developed antibody response to p28 at week 26.

Disposition of E11s Monkeys:

Necropsies on monkeys 93D, 107D, 67D (10^{-4} inoculum), 1D (10^{-5} inoculum), 94D (10^{-6} inoculum), and 116D (uninfected control) were performed on November 14, 1990, by Dr. Ribas and his team according to the protocol provided by Dr. Wohlheiter. On December 21, 1990, heparinized whole blood and serum samples were collected from animals 111D, 35D, 9D (10^{-5}), 87D, 3D, 83D (10^{-6}), and 39D, 30D, 103D (10^{-9}). In addition, lymph node biopsies were taken from 30D, 39D, and 103D (10^{-9}) for Dr. Ribas for pathological analysis.